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79. Genkichi Ohta, Osamu Nagase, Yasuhiro Hosokawa, Hiroaki Tagawa, and Masao Shimizu : Investigations on Pantothenic Acid and Its Related Compounds. III.*1 Chemical Studies. (2). Synthesis of Di-D-pantothenoyl-L-cystine.

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Synthesis of L-N,N′-diacylcystine (VI) from L-cysteine and nitrile (II) by using thiazoline (III) as an intermediate is described.

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It was proposed earlier by Novelli, *et al.*[1] that D-pantothenoyl-L-cysteine (Vb) might be the precursor of D-pantetheine (VIIb) in the biosynthesis of coenzyme A from pantothenic acid, but the step from Vb to VIIb was later questioned by Brown.[2] Because of its biochemical significance, the compound Vb or the corresponding disulfide (VIb) was prepared by several routes based on the general method of peptide synthesis.[3] The previous paper of this series described a new synthesis of D-pantethine which utilized 2-substituted 2-thiazoline as a key intermediate.[4] The thiazoline compound was obtained by the reaction of D-pantothenonitrile (Ib) with cysteamine and subsequently hydrolyzed to yield D-pantetheine. The method seemed to be applicable to the synthesis of Vb by using L-cysteine (II) in place of cysteamine. The present investigation was undertaken to examine further the scope of this thiazoline method and to prepare VIb for biochemical studies.

According to the results from the synthesis of firefly luciferin,[5] reaction of optically active cysteine with a nitrile at pH 8 proceeded without racemization giving a 4-carboxy-2-thiazoline derivative. In the case of reaction of L-cysteine with alkoxymethylnitriles, however, the optical activity of the resultant thiazolines was not reported.[6] In order to examine the maintenance of optical activity, our experiment was started with L-cysteine (II) and acetonitrile (Ia). Refluxing of an ethanolic solution of the two compounds in the presence of one molar sodium ethoxide afforded sodium L-2-methyl-2-thiazoline-4-carboxylate (IIIa), $[\alpha]_D$ +77.8°, which exhibited an ultraviolet absorption consistent with the thiazoline structure.[4] Reaction between the two compounds was effected when alkali was present and racemization was not observed by the use of one equimolar alkali. In the reaction without alkali, formation of the thiazoline ring, followed by gradual decomposition, was suggested by the ultraviolet spectrum and the thiazolinecarboxylic acid was not obtained. The 2-thiazolines are known to be unstable under mild acidic conditions,[4,7] and IIIa was isolable as its sodium salt. That the free acid was unstable was indicated by the sequel described

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1) *a*) L. Levintow, G.D. Novelli : J. Biol. Chem., **207**, 761 (1954). *b*) M.B. Hoagland, G.D. Novelli: *Ibid.*, **207**, 767 (1954).
2) G.M. Brown : J. Biol. Chem., **234**, 370 (1959).
3) J. Baddiley, A.P. Mathias : J. Chem. Soc., **1954**, 2803.
4) M. Shimizu, G. Ohta, O. Nagase, S. Okada, Y. Hosokawa : This Bulletin, **13**, 180 (1965).
5) E.H. White, F. McCapra, G.F. Field : J. Am. Chem. Soc., **85**, 337 (1963).
6) H. Baganz, J. Domashke : Chem. Ber., **95**, 1842 (1962).
7) Cf. *a*) H.A. Smith, G. Gorin : J. Org. Chem., **26**, 820 (1961). *b*) G.L. Schmir : J. Am. Chem. Soc., **87**, 243 (1965).

below. The L-compound was readily racemized by treatment with alkali and the slight excess of alkali in the condensation reaction caused racemization of the product. The L-compound was converted to its methyl ester (IVa) which was identified with an authentic sample.[7]

The hydrolytic fission of the C_2-S linkage of the thiazoline ring was carried out under weakly acidic conditions as reported previously.[4] A solution of the sodium salt (IIIa) was adjusted to pH 5.5 which required about 0.5 equimolar hydrochloric acid and the solution was warmed at 55°. By this treatment IIIa was converted to N-acetyl-L-cysteine (Va), $[\alpha]_D$ +5°, which, although it had a small rotation value, was distinguished by its melting point from the corresponding racemic compound. Oxidation of Va with hydrogen peroxide, followed by esterification with diazomethane, afforded the dimethyl ester of N,N'-diacetyl-L-cystine (VIa), $[\alpha]_D$ −110.5°, identical with an authentic sample.[8] The optical activity proved to be unaffected under above conditions.

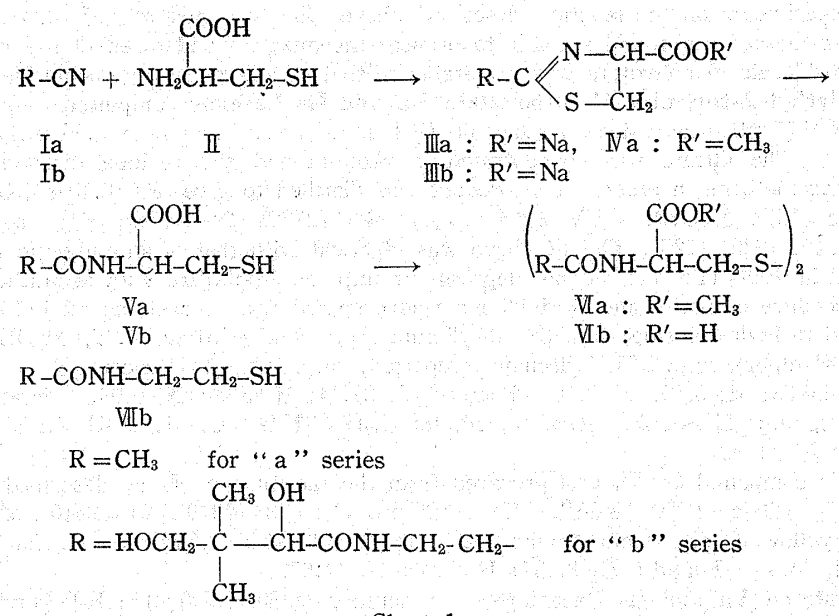

Chart 1.

According to these results, di-(D-pantothenoyl)-L-cystine (VIb) was prepared similarly. Condensation of D-pantothenonitrile (Ib) with L-cysteine in an ethanolic solution in the presence of one equimolar sodium ethoxide yielded sodium salt of the thiazolinecarboxylic acid (IIIb), $[\alpha]_D$ +30°, which revealed an ultraviolet spectrum characteristic of the thiazoline ring. The compound was hydrolyzed at initial pH of 5.1 until the ultraviolet absorption of IIIb disappeared. The product, without isolation, was oxidized with hydrogen peroxide to give VIb which was isolated as its barium salt and identified with an authentic sample prepared by the known method.[3] The over-all yield was 63.5%.

Experimental

Melting points are uncorrected. UV spectra were measured on a Hitachi EPU-2 spectrophotometer and IR spectra on a Hitachi EPI-2.

Sodium L-2-Methyl-2-thiazoline-4-carboxylate (IIIa) and Its Racemic Compound——a) To a solution of L-cysteine hydrochloride monohydrate (5.0 g., 0.0285 mole) in EtOH (35 ml.) was added an ethanolic sodium ethoxide solution (1.31 g. of Na in 35 ml., 0.057 mole) and the mixture was stirred thoroughly. Acetonitrile (1.40 g., 0.034 mole) was then added and the mixture was refluxed under N_2 for 8 hr. After cooling, the

8) H. Heymann: J. Am. Chem. Soc., **81**, 5125 (1959).

insoluble material was filtered, the filtrate was concentrated in vacuum and the residue was crystallized from EtOH to give a product (1.75 g.) melting at 198~209°. Recrystallization from the same solvent gave IIIa as hygroscopic needles (1.43 g.), m.p. 214~216° (decomp.), $[\alpha]_D$ +77.8° (c=1.08, H_2O). UV λ_{max}^{EtOH} mμ (ε): 235 (2760), 247 (2390). $\lambda_{max}^{1N\,HCl}$ mμ (ε): 261 (5720). IR ν_{max}^{KBr} cm^{-1}: 3350, 1630, 1600, 1400, 1280, 1215, 1170, 1130, 1070, 1020, 960, 860, 800, 690. *Anal.* Calcd. for $C_5H_6O_2NSNa$: C, 35.92; H, 3.59; N, 8.37; S, 19.14; Na, 13.75. Found: C, 35.76; H, 3.69; N, 8.21; S, 19.03; Na, 13.75.

b) In a similar manner, L-cysteine hydrochloride monohydrate (3.52 g., 0.02 mole) in EtOH (20 ml.) was treated with sodium ethoxide in EtOH (from 0.97 g. of Na in 20 ml., 0.042 mole) and then with acetonitrile (0.99 g., 0.024 mole) at reflux temperature for 8 hr. The insoluble material was filtered and extracted with hot MeOH (20 ml.). Evaporation of the methanolic solution and crystallization of the residue from MeOH gave prisms (0.66 g.), m.p. 238~242°. From the filtrate of the reaction mixture, after evaporation and crystallization from MeOH, a further crop was obtained (total 1.34 g.). Recrystallization from 90% EtOH gave the racemic compound of IIIa (1.02 g.), as plates, m.p. 243~245° (decomp.), $[\alpha]_D$ ±0° (c=0.80, H_2O). IR ν_{max}^{KBr} cm^{-1}: 1627, 1597, 1390, 1260, 1220, 1158, 1120, 1062, 1020, 964, 840, 680. *Anal.* Calcd. for $C_5H_6O_2NSNa$: C, 35.92; H, 3.59; N, 8.37. Found: C, 35.72; H, 3.60; N, 8.47.

The same compound was obtained by heating a solution of IIIa in EtOH with sodium ethoxide or in 90% MeOH with NaOH, whereas IIIa was recovered on heating the solution without alkali.

In repeated experiments on the reaction described above, the presence of 1.0~0.90 equimolar sodium ethoxide to free L-cysteine was found suitable to prevent racemization and to effect the reaction. The use of L-cysteine hydrochloride monohydrate was preferable to that of anhydrous hydrochloride.

Methyl L-2-Methyl-2-thiazoline-4-carboxylate (Va) and Its Racemic Compound——a) To a solution of IIIa (0.200 g.) in EtOH (5 ml.) was added an ethanolic HCl solution (7.85 mg./ml.; 5.56 ml.) and the separated NaCl was filtered. The filtrate was concentrated in vacuum and the residue dissolved in MeOH was methylated with diazomethane in ether. The product was distilled to give IVa (0.10 g.), b.p. 65~69°/3 mm. Hg, $[\alpha]_D$ +151° (c=1.20, MeOH). UV λ_{max}^{MeOH} mμ (ε): 234 (2720), 246 (2500), $\lambda_{max}^{1N\,HCl}$ mμ (ε): 261 (5450). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1740, 1625, 1225. The IR curve was identical with that of an authentic sample (see below). The picrate melted at 109~112° and did not depress the m.p. on admixture with an authentic sample.

b) By the procedure of Smith and Gorin[7a] IVa was prepared from L-cysteine methyl ester hydrochloride and ethyl acetoimidate hydrochloride; b.p. 68~69°/3 mm. Hg, $[\alpha]_D$ +151° (c=1.37, MeOH). Hydrochloride: needles (from MeOH-ether), m.p. 151.5° (decomp.) (reported, m.p. 143~144° (decomp.)).[7a] *Anal.* Calcd. for $C_6H_{10}O_2NSCl$: C, 36.83; H, 5.15; N, 7.16. Found: C, 37.14; H, 5.09; N, 6.90. Picrate: yellow prisms (from AcOEt-ether), m.p. 110~112°. *Anal.* Calcd. for $C_{12}H_{12}O_9N_4S$: C, 37.11; H, 3.12; N, 14.43. Found: C, 36.95; H, 3.10; N, 13.89.

c) The racemic compound of IVa was prepared from the racemate of IIIa as described in a); b.p. 75~78°/4 mm. Hg, $[\alpha]_D$ ±0° (c=1.30, MeOH). UV λ_{max}^{MeOH} mμ (ε): 234 (2940), 248 (2940), $\lambda_{max}^{1N\,HCl}$ mμ (ε): 261 (5620). Picrate: yellow needles (from AcOEt-ether), m.p. 114~116°. *Anal.* Calcd. for $C_{12}H_{12}O_9N_4S$: C, 37.11; H, 3.12; N, 14.43. Found: C, 37.37; H, 3.24; N, 14.67.

L-N-Acetylcysteine (Va) and Its Racemate——A solution of IIIa (0.500 g.) in H_2O (4 ml.) was adjusted to pH 5.5 with 1*N* HCl (1.3 ml.), heated under N_2 at 55° for 30 min., cooled and filtered through a column of "Amberlite IR 120 (H^+)" (2 ml.). The column was washed with H_2O and the combined effluent was evaporated in vacuum. Crystallization of the residue (0.498 g.) from AcOEt yielded Va as granules, m.p. 107.5~108°, $[\alpha]_D$ +5° (c=3.0, H_2O), (reported, m.p. 111°, $[\alpha]_{546}$ +6.3° (H_2O),[9] m.p. 107~107.5°[7a]). *Anal.* Calcd. for $C_5H_9O_3NS$: C, 36.80; H, 5.56; N, 8.58. Found: C, 36.89; H, 5.35; N, 8.61.

The DL-compound was prepared similarly from the DL-compound of IIIa, m.p. 124.5~125.5°, $[\alpha]_D$ ±0° (c=3.0, H_2O). *Anal.* Calcd. for $C_5H_9O_3NS$: C, 36.80; H, 5.56; N, 8.58. Found: C, 37.18; H, 5.69; N, 8.61.

L-N,N'-Diacetylcystine Dimethyl Ester (VIa)——As described above, IIIa (0.500 g.) in H_2O (4 ml.) was hydrolyzed with HCl, and the resultant solution was adjusted to pH 8.0 with $NaHCO_3$. To this was added dropwise 3% H_2O_2 solution until the reaction mixture no longer colored with sodium nitroprusside reagent. The mixture was adjusted to pH 1.8 with 1*N* HCl, concentrated in vacuum and extracted with AcOEt. The AcOEt solution was evaporated in vacuum and the residue, dissolved in MeOH, was treated with diazomethane in ether. Crystallization of the product from AcOEt gave VIa as needles, m.p. 123.5~126.5° undepressed on admixture with an authentic sample (see below), $[\alpha]_D$ −110.5° (c=1.1, MeOH).

An authentic sample of VIa was prepared from L-cystine dimethyl ester and acetyl chloride by the procedure of Heymann,[8] and crystallized from AcOEt, m.p. 126.5~128.5°, $[\alpha]_D$ −113.5° (c=1.1, MeOH), (reported, m.p. 125~129°, $[\alpha]_{546}$ −86.1° (H_2O)).

Sodium L-2-(2-D-Pantamidoethyl)-2-thiazoline-4-carboxylate (IIIb)——To a solution of L-cysteine hydrochloride monohydrate (12.0 g.) in EtOH (250 ml.) was added a solution of sodium ethoxide in EtOH (3.14 g. of Na in 250 ml.) and the mixture was stirred at room temperature. D-Pantothenonitrile (Ib) (12.48 g.) in EtOH (100 ml.) was then added and the mixture was refluxed under N_2 for 8 hr. The insoluble material was filtered and the filtrate was concentrated in vacuum. The residue was extracted with hot AcOEt and

9) N. W. Pirie, T. S. Hele: Biochem. J., **27**, 1716 (1933).

the insoluble material was collected; this was practically pure IIIb (20.5 g.). For further purification, the product (2.00 g.) was dissolved in EtOH (50 ml.) and chromatographed through a column of cellulose powder–charcoal (3:1, 20 g.). The column was developed with EtOH and fractions of each 25 ml. were collected. Fractions nos. 5～16 were combined and evaporated and the residue was dissolved in EtOH. Addition of AcOEt to the solution gave an amorphous precipitate (1.49 g.) which was re-precipitated from an ethanolic solution by adding ether to give pure IIIb, $[\alpha]_D$ +30.3°(c=1.0, H_2O). UV λ_{max}^{EtOH} mμ (ε): 234 (2180), 248 (1890), $\lambda_{max}^{1N\,HCl}$ mμ (ε): 265 (4140), IR ν_{max}^{KBr} cm^{-1}: 3330, 2860, 1651, 1603, 1531, 1396, 1266, 1077, 1042, 982, 910. Paperchromatography: Rf 0.72, by ascending technique with 70% EtOH on "Toyo filterpaper No. 50." *Anal.* Calcd. for $C_{12}H_{19}O_5N_2SNa \cdot \frac{1}{2}H_2O \cdot \frac{1}{2}C_2H_5OH$: C, 43.56; H, 6.47; N, 7.82; Na, 6.42; C_2H_5O, 6.29. Found: C, 43.42; H, 6.81; N, 7.76; Na, 6.30; C_2H_5O, 7.29.

N,N'-Di-D-pantothenoyl-L-cystine (VIb)——A solution of IIIb (2.00 g.) in H_2O (15 ml.) was adjusted to pH 5.1 with 1*N* oxalic acid and heated at 55～60° for 40 min. The solution was cooled with ice-water, adjusted to pH 8.0 with 14% NH_4OH, and oxidized with 3% H_2O_2 until it no longer colored sodium nitroprusside reagent. The solution was filtered through a column of "Amberlite IR 120 (H^+)" (15 ml.) and the column was washed with water (100 ml.). The eluted solution was adjusted to pH 6.9 with aqueous $Ba(OH)_2$ solution and the separated barium oxalate was filtered. The filtrate was evaporated in vacuum and the residue (1.8 g.) was dissolved in small quantities of MeOH. Addition of ether gave a precipitate which was collected. This was purified by precipitation from MeOH solution with EtOH and then from MeOH with ether to give the barium salt of VIb, amorphous powder, m.p. 200～215°(decomp.), $[\alpha]_D$ −75°(c=1.0, H_2O). IR ν_{max}^{KBr} cm^{-1}: 3325 (OH, NH), 1658, 1643, 1535, 1285 (CONH), 1594, 1396 (COO^-). Paperchromatography: Rf 0.56 by ascending technique with *n*-BuOH–AcOH–H_2O (4:1:5, Upper phase) on "Toyo filterpaper No. 50." This was identical with an authentic sample (see below) in all respects (IR, paper chromatography, and thin-layer chromatography). *Anal.* Calcd. for $C_{24}H_{40}O_{12}N_4S_2Ba \cdot 2H_2O$: C, 35.41, H, 5.45; N, 6.88. Found: C, 35.46; H, 5.58; N, 7.13.

After drying in vacuum at 80° an anhydrous sample was obtained. *Anal.* Calcd. for $C_{24}H_{40}O_{12}N_4S_2Ba$: C, 37.05; H, 5.18; N, 7.20; Ba, 17.65. Found: C, 36.99; H, 5.36; N, 6.43; Ba, 17.30.

An authentic sample of VIb was prepared by the procedure of Baddiley and Mathias,[3] $[\alpha]_D$ −74.5°(c=0.96, H_2O).

The barium salt of N-D-pantothenoyl-L-cysteine (Vb) was prepared by reduction of the barium salt of VIb with aqueous β-mercaptoethanol for 3 hr., and by precipitation from MeOH with ether. $[\alpha]_D$ −14°(c=2.0, H_2O). Paper chromatography: Rf 0.73 in *n*-BuOH–AcOH–H_2O (4:1:5, Upper phase). *Anal.* Calcd. for $C_{12}H_{21}O_6N_2SBa_{1/2} \cdot H_2O$: C, 35.32; H, 5.68; N, 6.87. Found: C, 35.09; H, 6.07; N, 6.98.

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